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(54) Title: IMMUNOPROTECTIVE MEMBRANE

(57) Abstract

A size exclusion membrane, particularly an immunoprotective membrane, is formed by filling the pores of a supporting membrane with a hydrogel and cross-linking the hydrogel in a hydrated state. The supporting membrane is a porous membrane having pore size of less than 20 μm and a tortuosity greater than 1 and preferably greater than 1.2. In a preferred embodiment, the supporting membrane is an open cell polyethersulfone wherein the pores are filled with a hydrated polyvinyl alcohol hydrogel which is cross-linked with, for example, glutaraldehyde while in a hydrated state. This can be used in a variety of different applications such as drug delivery, in vitro and in vivo filtration, and, for example, protection of pancreatic islet cells to provide a bio-artificial pancreas.

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IMMUNOPROTECTIVE MEMBRANE

Background of the Invention

Any foreign substance which is introduced into the body is generally subjected to an immune system reaction. Many substances are basically inert and are not recognized and/or attacked by the immune system; however, most living organic matter or matter derived therefrom, with the exception of certain matter introduced through the gastro-intestinal system, will be attacked by the immune system unless some type of preventive measure is taken.

It is frequently desirable to introduce a medical device into the body for relatively long periods of time. This might be for drug delivery or other applications such as artificial organs. One particular artificial organ of interest is the bio-artificial pancreas. It is believed that pancreatic islet transplantation may offer an ideal endocrine replacement therapy for patients with *diabetes mellitus*. Two major problems associated with such islet transplantation is recurrence of the original

disease in the case of employing unprotected islet cells, and an immune rejection of foreign tissue.

Immunosuppressive therapy can be used in association with islet transplantation, but this has serious side effects.

Both for drug delivery systems and for cellular transplantation, it has been suggested to protect the foreign substance or transplanted cells using a membrane which will permit the flow of smaller molecules necessary for cellular functions while, at the same time, excluding larger molecules and cells associated with the immune system. The ideal situation is a membrane which is suitable for use in an aqueous environment wherein the membrane will permit the passage of smaller molecules, i.e. less than about 20,000 Daltons, such as glucose, but will exclude larger molecules, i.e. greater than about 60,000 Daltons, such as the immunoglobulin molecules and other humoral components in the immune system. These are generally on the order of 50 angstroms in diameter or greater.

Inoue (*Pancreas*, Vol. 7, No. 5, pp. 562-568), has proposed the use of a polyvinyl alcohol membrane as an immunoprotective membrane for entrapment of islet cells. However, the polyvinyl alcohol membrane is produced by simply bonding crosslinked polyvinyl alcohol to a polyester mesh tube having openings of about 60 μm. The produced film, however, is generally too weak to be successfully implanted and, once implanted, to withstand long-term internal stresses within the body. This same product is discussed in *Cell Transplantation*, Vol. 3, Supp. 1,

pp. S19-S21 (1994) and in *Transplantation Proceedings*, Vol. 27, No. 1 (Feb. 1995), pp. 619-621.

Other artificial pancreases are described in *Fournier* U.S. Patents 5,387,237 and 5,425,764.

Summary of the Invention

Accordingly, it is an object of the present invention to provide a biocompatible, immunoprotective membrane which is an extremely hydrophilic membrane which allows free transport of small molecules and, at the same time, excludes larger molecules of the immune system such as the immunoglobulins and other cellular components of the immune system such as T-cells and the like which are part of the immune system.

Further, it is an object of the present invention to provide such a membrane that has sufficient strength to withstand the pressure differentials associated with implantation and cell loading within the body.

These objects and advantages are achieved by coating and/or impregnating a micro-porous, supportive membrane with a hydrogel and cross-linking the hydrogel, wherein the porous, supporting membrane has a pore size and internal surface area per volume of gel which holds the gel in position. Preferably the supporting membrane is a hydrophilic membrane, and in a preferred embodiment is an open-celled foam material having thickness of 10 to about 200 μm. One preferred material is an open-celled polyethersulfone material such as

that produced by Gelman Sciences and sold under the name Supor®. The pore size should be from 0.01 μm, preferably 0.2 μm which would serve as a barrier to cellular components of the immune system, up to 10-20 μm.

The objects and advantages of the present invention will be further appreciated in light of the following detailed description.

Brief Description of the Drawings

FIG. 1 is a graph comparing membrane permeability of a membrane made according to the present invention and commercially available immunoprotective membranes.

FIG. 2 is a graph comparing the effective membrane diffusivity of a membrane made according to the present invention and commercially available immunoprotective membranes.

FIG. 3 is a graph showing permeability of an implanted membrane over a 6-month period.

Detailed Description

The present invention is an immunoprotective membrane which comprises a supporting membrane coated or impregnated with a cross-linked hydrogel. The cross-linked hydrogel is designed to permit passage of water and smaller molecules such as glucose and insulin, and prevent larger molecules such as the immunoglobulins and cellular components such as T-cells of the immune system from passing through the hydrogel.

The supportive membrane will be a biocompatible polymeric membrane which will not break down when implanted within the body, and which has a pore size of from 50 Å to about 50 μm. At a pore size less than 50 Å the supportive membrane itself would physically exclude immunoglobulins and therefore pore size any smaller than this is unnecessary. Preferably, the pore size will be from about 0.01 μm to about 20 μm and have a void volume of at least about 50% and preferably greater than 80%, preferably the membrane will have a pore size of 0.2 to 10 μm, although 0.2 μm is preferred since it is sufficient to block cellular immune components.

The membrane further should have a thickness that provides acceptable solute permeabilities for molecules less than about 20,000 Daltons. This acceptable permeability range is dependent on the types of cells being protected, their metabolic needs, the desired therapeutic response, and the overall device configuration.

Permeability of any solute may be defined as the ratio of the effective diffusivity and the membrane thickness. The effective diffusivity is dependent on the solute size, and hence its diffusivity in water, and the nature of the gel system used, which is under experimental control. Also, the membrane thickness is a separately controllable variable. The ratio of effective diffusivity to the diffusivity in water ($D_{effective}$ / D_{water}) defines the ease with which a particular solute can pass through the membrane. Certainly, for a small solute, this ratio ideally will approach one and can never be greater than one; however, for a larg r solute like a component

of the immune system, this ratio should be very small. At molecular weights greater than about 100,000 Daltons, the ratio of $D_{effective}$ / D_{water} should be extremely low in order to minimize penetration through the membrane of immunoglobulins and components of the complement system. For solutes of 100,000 Daltons or higher, the ratio of $D_{effective}$ / D_{water} should be less than 0.01 and more preferably less than 0.001. A preferred membrane will have a ratio $D_{effective}$ / D_{water} from 10^{-6} to 0.001.

The thickness of the supporting membrane will generally be from 10 microns to 500 microns or more while maintaining the permeability of various solutes within the desired range. A preferred thickness is from 20 microns to 200 microns. The permeability of a freely permeable solute such as glucose will generally be from 5 X 10^{-5} to 5 x 10^{-3} cm/sec. A preferred glucose permeability would be at least 10^{-4} cm/sec. For a 100,000 Dalton solute that is generally restricted by the membrane, the permeability will be from 10^{-8} cm/sec to 10^{-6} cm/sec. A preferred permeability for this size solute would be less than 5 x 10^{-7} cm/sec.

For practicing the present invention, it is preferable that the supporting membrane be a relatively hydrophilic membrane. A hydrophilic support is preferred since it easily draws the aqueous hydrogel solution into its porous structure. A hydrophobic structure would not as easily draw into its porous structure the hydrogel material. However, a hydrophobic support can be used by treating its surface first with any number of chemical prewetting agents of low surface tension

such as alcohol. Also, higher pressures may be used to force the hydrogel material into the pores of the hydrophobic support structure.

Hydrophilicity of a membrane can be defined by the water contact angle wherein the angle of contact of water with the nonporous surface as the support material is measured. It is preferred that the membrane have a hydrophilicity defined by this test of at least about 20 dynes/cm.

For use in the present invention the membrane must be able to hold or support the hydrogel at elevated pressures, i.e., those pressures which would be encountered in the body. This pressure will be based on the physical distance between the right atrium and where the device is implanted. If the device is implanted in the abdomen, the distance will be about 4 inches for a child and the pressure will be about 0.13 psi. For an adult, this pressure will be about 0.4 psi. Thus, the membrane must hold the hydrogel at a pressure of 0.13 psi and preferably 0.4 psi (Gauge) to about 4 psi, and more preferably at least 1.5 psi. To accomplish this, the pores should have sufficient internal surface area to support the gel. Further, the pore size should be less than about 50 μm and preferably less than 20 μm. Preferably, the pores will have a tortuosity greater than 1. Tortuosity is the ratio of a typical pore path length to the thickness of the membrane. If the membrane is a mesh such as disclosed in *Inoue*, the tortuosity, by definition, is 1. If pores do not extend straight through the membrane, the tortuosity is greater than 1. According to the present invention, it is preferable that the tortuosity

be greater than 1, and preferably should be about 1.2 to about 4.0 with 1.4 to 3.0 preferred. The internal surface area pore size and tortuosity all combine to enable the membrane to hold the hydrogel at these elevated pressures.

The chemical composition of the supporting membrane can vary. Of course, it must be biologically acceptable and inert and preferably hydrophilic. Useful materials include the polyesters such as the polyacrylates and polymethacrylates, poly(ethylene terephthelate), the polyamides, polyacrylonitriles, polyanhydrides, poly(orthoesters), low density polyethylene, high density polyethylene, polypropylene, polyvinyl chloride, polystyrene, polyvinylpyrolidone, poly(lactide-co-glycolide), poly(etherurethane), poly(etherurethane urea) and polyethersulfones which are preferred.

The structure of the material can vary from compacted, non-woven webs to cellular structure, both open-cell and closed-cell. One preferred physical structure is an open-celled foam structure. One such material which is a culture filtration membrane can be purchased from Gelman Sciences and is sold under the brand name Supor®. This is an open cellular polyethersulfone having an average pore size of 0.2 microns.

The immunoprotective membrane is formed by bonding either on the surface or within the pores to the supportive membrane a hydrogel which is subsequently cross-linked, preferably while in the hydrated condition. Hydrogels are cross-linked polymer networks which

have the ability to swell in water or aqueous solvent systems. The polymer structure is able to retain the solvent forming a swollen gel phase and, in cross-linked systems, will not dissolve regardless of the amount of solvent present. There are a number of different hydrogels which can be used in the present invention. The hydrogels can be of natural or synthetic organic or inorganic material. They are normally made of water soluble backbone materials which are rendered insoluble by the introduction of covalent crosslinks. Common hydrogels include addition polymers of hydroxy alkyl(meth)acrylates, methyl vinyl ether, (meth)acrylamide, N-vinyl pyrrolidone, (meth)acrylic acid and its salts, N-vinyl and C-vinyl pyrridines and salts thereof with poly(meth)acrylates such as glycol dimethacrylate. There may also be used crosslinked natural polymers such as collagen, glycosaminoglycans, or starch and cellulose derivatives, and crosslinked synthetic polymers such as polyvinyl alcohol may be used.

Suitable cross-linked materials can be prepared by reacting poly(ethylene oxide) or poly(ethylene glycol) with a polyol (e.g., 1,2,6-hexantriol) and a polyisocyanate (e.g., diphenyl-methane 4,4'-diisocyanate). Further, there may be used insoluble domains (block copolymers of e.g. polyethylene oxide with water-insoluble urethane blocks) or materials rendered insoluble by entanglement crosslinking (high molecular weight poly(ethylene oxides) with divinylbenzene or by crystallinity (cellulosic materials).

The preferred hydrogel is polyvinyl alcohol hydrogel crosslinked with gluteraldehyde.

For use in the present invention, the water content of the hydrogel should be from about 60 to about 98%. The concentration of water in the hydrogel is a function of cross-linking. The water content and amount of cross-linking are inversely proportional. Therefore, by increasing cross-linking one decreases water content but, at the same time, strengthens the hydrogel.

The hydrogel is applied to the supporting membrane using any standard technique. One simple technique is to form an aqueous dispersion of the polymer and soak or dip the support membrane in the dispersion prior to crosslinking. The solution will migrate into the pores and fill the pores of the support membrane, in large part because of the hydrophilicity of the support membrane. The polymer solution can then be crosslinked within the membrane pore.

The following example describes the preparation of a hydrogel and coating or filling of a polyethersulfone open celled foam membrane.

Example:

The PVA/GA/PES membrane is an effective semipermeable immunoisolation membrane system in which a glutaraldehyde (GA) crosslinked polyvinyl alcohol (PVA) hydrogel is incorporated into the void space of a highly permeable polyethersulfone (PES) support filter.

PVA Solution

An aqueous solution containing 3 wt% PVA, 0.083 wt% GA, and 0.1 N HCl is prepared as follows:

Reagent	Amount for 10 g Solution
Polyvinyl Alcohol 9,000-10,000 ave MW, 80% hydrolyzed, Aldrich #36,062-7	0.300 g
Glutaraldehyde a/k/a glutaric dialdehyde 50 wt% solution in water, Aldrich #34,085-5	15 µl
1.0 M HCl Solution	984 µl
Distilled Water	8.71 ml.

1. Dissolve the PVA in the water at room temperature with vigorous stirring.
2. Add HCl and mix.
3. Add glutaraldehyde and mix thoroughly.
4. Store at room temperature for up to 7 days.

Filter Treatment

Polyethersulfone filters (0.2µm Supor-200, Gelman Sciences #60300) are treated with the PVA solution as follows:

1. Filters are submerged in room temperature PVA solution until fully wetted.
2. Store wetted filters on polypropylene rack at 37° C/90% humidity for 24 hours.
3. Resubmerge the filters in room temperature PVA solution and store for an additional 24 hours at 37° C/90% humidity.

4. Submerge the filters a third time in room temperature PVA solution and store at 37° C/90% humidity for 18 hours.

5. Place the filters in boiling distilled water immediately upon removal from the constant temperature/humidity environment. Boil for 30 minutes.

6. Store membranes in distilled water or saline (0.9 wt% NaCl) until ready for use. Membranes should be kept in contact with water or saline at all times.

7. Sterilize membranes by autoclaving in distilled water or saline at 123° C for 20 minutes.

The coated membrane had a thickness of 154.9 ± 3.9 μm, a hydrogel water fraction of 86.0% ± 0.6% and a total water fraction of 64.7% ± 0.4%.

The concentration of the crosslinking agent, i.e., glutaraldehyde, controls the water concentration of the polyvinyl alcohol hydrogel. By varying the concentration from nearly 0 to about 0.8% glutaraldehyde, the water content of the hydrogel can be varied from 97% down to about 80%. Accordingly, it is preferred that the water content be maintained at from about 85% to about 97%. Accordingly, the glutaraldehyde concentration is established at about 0.1%.

The membranes formed according to the example were tested and compared with commercially available membranes. These comparisons are shown in FIGS. 1 and 2. The membranes were implanted in rats and the permeability tested for various periods of implantation over a period of 6 months. These results are shown in FIG. 3.

The membrane of the present invention can be formed in a variety of different shapes. It can be planar. It can be in the form of a tube or hollow fiber, or spiral wound configuration. It can also be used in conjunction with other devices to separate the materials including cells from biological molecules.

In further use of the present invention, the immunoprotective membrane can be folded upon itself and further physically clamped using plastic or stainless steel clamps to hold the sheets adjacent to each other to form an envelope. Alternately, they can be used in association with devices such as those disclosed in U.S. Patents 5,387,237 and 5,425,764. These can also be used in a variety of different applications such as in dialysis machines or other filtration devices or systems for separation or segregation of molecules. These could also be used in laboratory or industrial cellular growth and fermentation applications where it is desirable to separate various molecules based on size. Accordingly, the semipermeable membrane of the present invention can be used both in vivo and in vitro as a size exclusion membrane.

The membrane of the present invention has a number of different applications and of course can be modified by using different membranes and different hydrogels, depending upon the various desired applications. Accordingly, the invention itself should only be defined by the appended claims wherein we claim:

1. An immunoisolation membrane comprising a porous membrane film having pores extending through said film wherein said pores are filled with a hydrogel, said hydrogel cross-linked in a hydrated state, said pores having a diameter less than about 20 μm and an internal surface area effective to maintain said hydrogel bonded to said film.

2. The membrane claimed in claim 1 wherein said porous membrane is selected from the group consisting of polyesters, polyamides, polyethersulfones and polyurethanes.

3. The membrane claimed in claim 2 wherein said porous membrane has a foamed structure.

4. The membrane claimed in claim 3 wherein said membrane is an open cell foam.

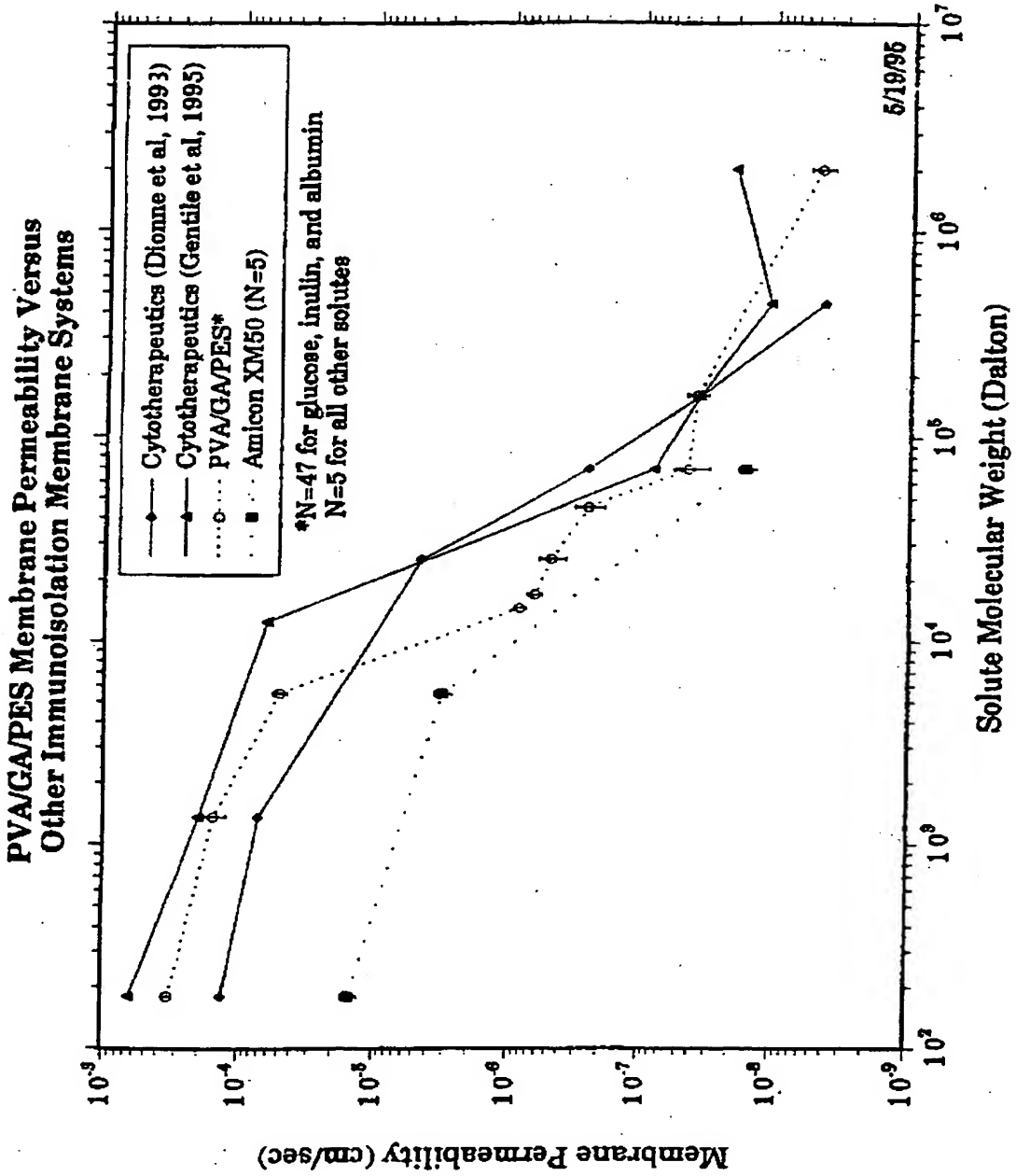
5. The membrane claimed in claim 4 wherein said porous membrane is a polyethersulfone.

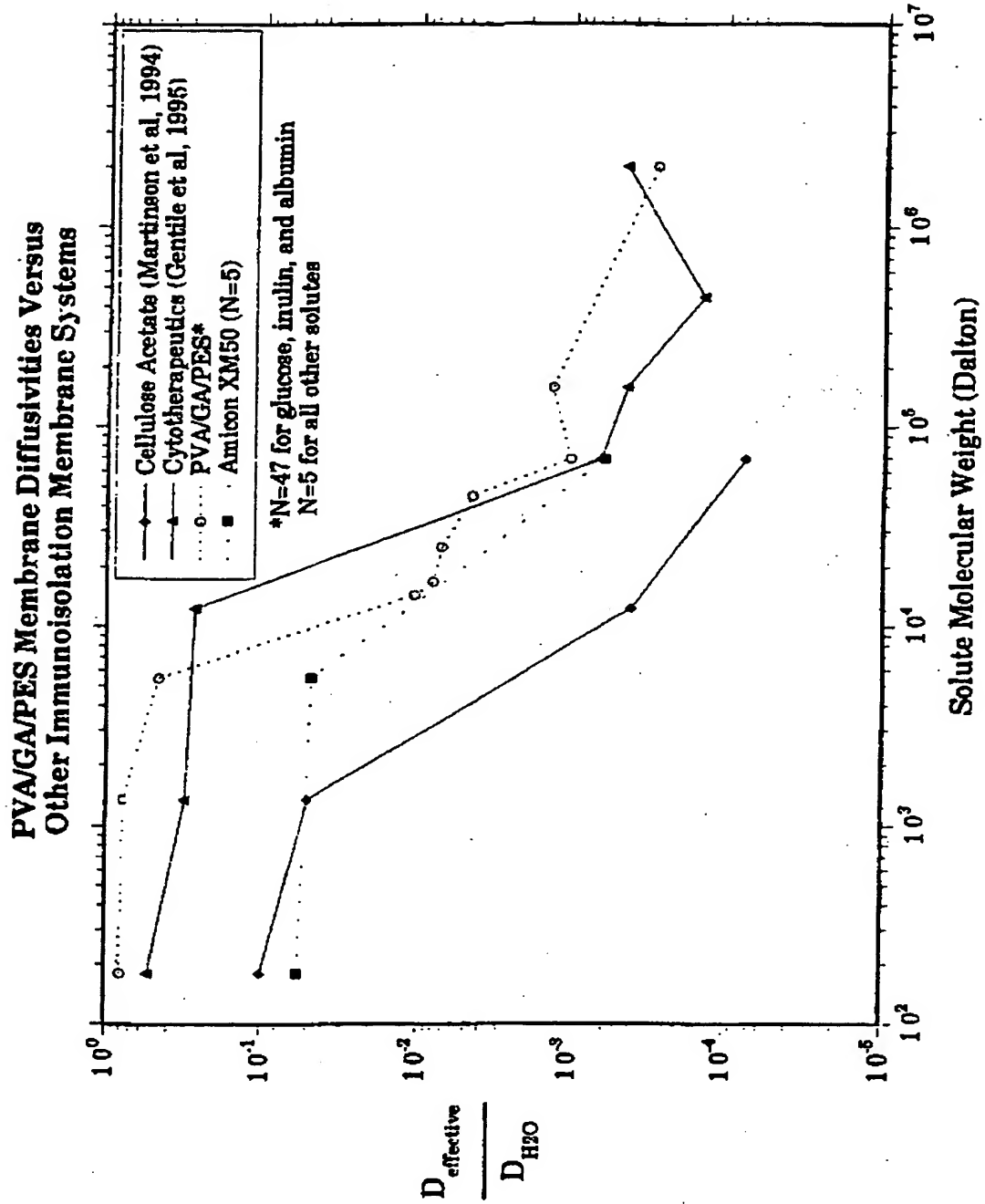
6. The membrane claimed in claim 1 wherein said hydrogel is polyvinyl alcohol.

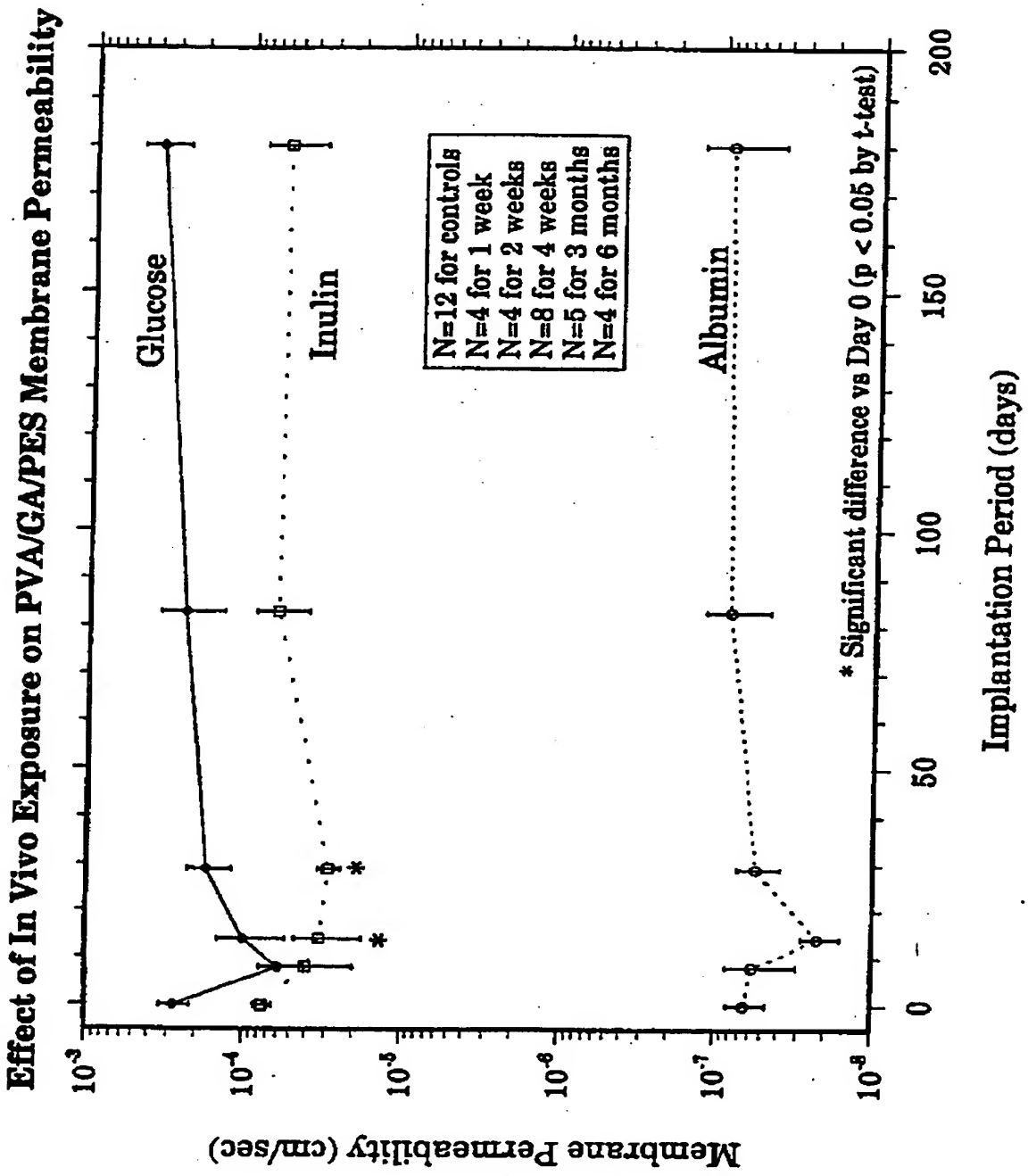
7. The membrane claimed in claim 6 wherein said hydrogel is polyvinyl alcohol crosslinked with gluteraldehyde.

8. The membrane claimed in claim 1 wherein said porous membrane has a pore size of from about 0.01 microns to about 20 microns.

9. An immunoisolation membrane comprising a supporting porous membrane having pores, wherein said porous membrane has an average tortuosity greater than 1, wherein said membrane is an open cell foam polyethersulfone, said pores filled with a hydrogel, said hydrogel comprising polyvinyl alcohol cross-linked in a hydrated state whereby said membrane excludes molecules greater than about 100,000 and does not exclude molecules having a molecular weight less than about 20,000.


PVA/GA/PES Membrane Permeability Versus
Other Immunoisolation Membrane Systems
Cytotherapeutics (Dionne et al, 1993)
Cytotherapeutics (Gentile et al, 1995)
PVA/GA/PES*
Amicon XM50 (N=5)
*N=47 for glucose, inulin, and albumin
N=5 for all other solutes
5/19/95
Membrane Permeability (cm/sec)
Solute Molecular Weight (Dalton)


PVA/GA/PES Membrane Diffusivities Versus
Other Immunoisolation Membrane Systems
Cellulose Acetate (Martinson et al, 1994)
Cytotherapeutics (Gentile et al, 1995)
PVA/GA/PES*
Amicon XM50 (N=5)
*N=47 for glucose, inulin, and albumin
N=5 for all other solutes
$D_{effective} / D_{H2O}$
Solute Molecular Weight (Dalton)


Effect of In Vivo Exposure on PVA/GA/PES Membrane Permeability
Glucose
Inulin
Albumin
N=12 for controls
N=4 for 1 week
N=4 for 2 weeks
N=8 for 4 weeks
N=5 for 3 months
N=4 for 6 months
* Significant difference vs Day 0 (p < 0.05 by t-test)
Membrane Permeability (cm/sec)
Implantation Period (days)
10^-3
10^-4
10^-5
10^-6
10^-7
10^-8
0
50
100
150
200

INTERNATIONAL SEARCH REPORT

International application N .
PCT/US96/17707

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :B01D 71/68, 71/38
US CL :210/500.27, 500.41; 436/824; 52/141, 142, 155
According to International Patent Classification (IPC) r to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 210/500.27, 500.41; 436/824; 52/141, 142, 155; 521/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,041,225 A (NORMAN) 20 August 1991, see columns 4, 5 and 6.	1-9
Y	US 5,104,729 A (STEDRONSKY) 14 April 1992, see columns 9-12.	1-9
Y	US 4,879,316 A (ALEXANDRATOS ET AL) 07 November 1989, see entire document.	1-9
Y	US 4,220,152 A (DRESBACK) 02 September 1980, see columns 9 and 10.	1-9
Y	US, 5,443,727 A (GAGNON) 22 August 1995, see columns 36-46.	1-9
Y	JP 59-62351 A (ASAHI CHEMICAL IND KK) 09 April 1984, see abstract.	1-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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